The Relationship Between Hollow Heart of Pea and Seed Electrolyte Loss, Disease Susceptibility, and Plant Growth

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ABSTRACT

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The effects of hollow heart of pea on seed electrolyte loss, seedling growth, and susceptibility to *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* were studied. Linear regression analysis of hollow heart severity and mean electroconductivity (EC) readings for seed in each hollow heart category were highly correlated in 12 pea cultivars. All regressions were significant at $P < 0.001$. EC readings for healthy seed varied considerably among varieties, but healthy seed averaged 36 μamps less than seed with severe hollow heart. When seed were separated into EC categories of 25–50, 55–65, 70–90, and 91–200 μamps, there was a highly significant relationship ($P < 0.001$) between plant growth parameters and EC category. When a second set of seed was divided into the same EC categories and then evaluated for degree of hollow heart, only the most severely affected seed were in the highest EC category. Separation of seed into severity categories is essential for obtaining consistent results on growth responses to hollow heart. Severe hollow heart increased susceptibility to root pathogens but not enough to affect seedling growth.

Additional key words: electrical conductivity, physiological disease, *Pisum sativum*, predisposition, root rot, seedling vigor.

Hollow heart of pea (*Pisum sativum* L.) is a physiological disorder that is characterized by a cavity on the adaxial surface of the cotyledons of germinating seed. Most of the research on hollow heart has been conducted in Scotland and New Zealand, but recently it was identified as a common disorder of seed peas in the United States (15,16). Seed are predisposed to hollow heart during maturation (13,14), but symptom development is dependent on moisture conditions during germination (13,16). Germination is not affected, but seed with the disorder are highly susceptible to rot by saprophytic fungi (6,13,16). The portion of the seed affected by hollow heart is unavailable as a nutrient source for the developing seedling (5). Still, effects of the disorder on seedling vigor are disputed (4–7,10).

Seedling vigor of peas has been associated with electroconductivity (EC) of seed steep water (1,11,13). Poor quality seed of low vigor exude more electrolytes than healthy seed from the same lot and, therefore, give higher EC readings (8). Because peas are hypogeal, increased exudation of electrolytes directly affects spermosphere microorganisms. Pea seed exudation has been shown to increase root rots caused by *Pythium ultimum* Trow and *Fusarium solani* (Mart) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans by providing available nutrients for the pathogen at the infection court (8,9). Because the portion of the cotyledon affected by hollow heart dies during germination (14), one could logically expect an increase in seed exudates. However, in evaluating the relationship between hollow heart and EC, researchers have disagreed. Reports of strong (18), weak (2), or no correlation (3,14,18) have been published. Techniques used to evaluate the relation between hollow heart and EC in these studies were usually similar to those prescribed by the International Seed Testing Association; i.e., 50 seed are soaked for 24 hr in 250 ml of distilled H_2O at 20 C, and then EC readings are taken on the steep water. This method is inadequate because affected and unaffected seed are bulked together and increased exudation from hollow heart seed could easily be buffered to a point of nondetection.

When one works with root-rotting organisms of pea, a disease index is frequently used to describe different levels of disease severity (8,9,17). Often peas can be infected by *F. solani* f. sp. *pisi*, but unless the infection is extensive and environmental conditions favor disease development, no effect on growth can be measured (17). To date, there has been considerable disagreement about the relationship of hollow heart, a physiological disease, to plant growth, EC, disease susceptibility, and field performance.

Although previous researchers have included seed lots with different percentages of hollow heart, none have actually established hollow heart severity categories. The failure to differentiate between degrees of hollow heart severity could explain much of the controversy in hollow heart research. Therefore, the first objective of this study was to establish hollow heart severity categories and then to evaluate the effects hollow heart has on plant growth and susceptibility to root disease. The second objective was to evaluate the usefulness of an automatic seed analyzer in determining the relationship between hollow heart severity categories and EC.

MATERIALS AND METHODS

Electroconductivity and hollow heart determinations. Based on results of a previous study (16), 12 pea cultivars that differed in percent hollow heart were selected for EC studies. EC readings for individual seeds were obtained as previously described (8,16) using the ASAC-1000 automatic seed analyzer (Neogen Food Tech Corp., Lansing, MI). One hundred seed per replication were placed in individual compartments and soaked in glass distilled water at 22 ± 1 C for 24 hr. All EC readings were displayed as μamps. There were five replicates for each cultivar.

After EC readings, seed were taken from the soaking trays and placed in numerical order on wet germination papers. These were rolled and incubated at 20 C for 6 days. After incubation, the cotyledons of each germinated seed were separated and checked for the presence or absence of hollow heart. Healthy, symptomless seed were placed in a separate category. Those with hollow heart were separated into five categories ranging from only a slight indention or dimple to severe cavitation in which >75% of the cotyledon was affected by the disorder. Seed with minor dimples, approximately 1 mm, on the adaxial surface of the cotyledon and seed with small indentions plus a crack, were scored as D1 and D2, respectively. The remaining three categories C1, C2, and C3 contained cavitated seed of increasing severity. Severity was based on diameter and depth of cavities and degree of discoloration or bleaching of tissue. Seed with comparatively small, shallow cavities with little or no tissue bleaching were rated C1. C2 seed had relatively wide cavities, which were much deeper than C1 cavities. Affected tissue was usually normal in appearance. A C1-sized cavity with discolored tissue would also be rated as a C2. Cavities of C3 seed were usually discolored and encompassed the majority of the cotyledonary tissue (Fig. 1). With all categories, cracks through the affected tissue were often present. However, some cultivars had more cracking associated with the disorder than others and, therefore, cracking was not used, except in D2, as a category characteristic.

For purposes of regression, the healthy seed and five hollow heart categories were assigned a numerical value 1–6, corresponding to healthy through C3 seed. A linear regression was made of hollow heart severity category 1–6 on EC means of seed in each hollow heart category. This study was repeated twice. Fewer replicates were evaluated in the first test, but results were the same.

Cultivar OSU-605 was further evaluated. Four EC categories were established, 25–50, 55–65, 70–90, and 91–200 μamps. After EC readings were taken, seed were separated into EC categories and then evaluated for hollow heart as described. There were five replications of 100 seed each. The purpose was to determine which hollow heart severity categories predominated in each specific EC category. These EC categories were selected on the basis of unpublished preliminary studies by the author.

Growth studies. EC values were obtained for individual seed of cultivar OSU-605. Seed were then separated into one of four EC categories, 25–50, 55–65, 70–90, or >90 μamps. There were five replicates of 25 seed per replicate for each category. Seed were planted in the greenhouse in cones containing a nonsterile Ritzville fine sandy loam peat mix 4:1, v/v. Cones were arranged in a randomized block. Seedlings were harvested 3 wk after planting and plant height, dry top weight, leaf area, and dry root weight were measured. These growth data were analyzed using analysis of variance (ANOVA), followed by regressions of plant growth parameter means vs. EC category means of 37, 60, 80, and 145 μamps.

Hollow heart and susceptibility to root rots. Seed of cultivar OSU-605 were rolled in moist germination paper and incubated at 20 C for 6 days. Cotyledons of germinated seed were gently pried apart just enough to determine hollow heart severity. If the tissue connecting the cotyledon to the emerging seedling tore, the seed would be discarded. Special care was taken not to damage the emerging root or epicotyl. The young seedlings were then separated into four categories: severe hollow heart (C2 and C3), healthy, healthy with one cotyledon removed, and healthy with both cotyledons removed. Cotyledons were surgically removed with a scalpel without further damaging the young plant. The treatments where one or both cotyledons were removed were included to evaluate how healthy seedlings would develop when a portion of the seed was removed. This was intended to simulate the unavailable portion of seeds severely affected by hollow heart. Seedlings were then planted into a Ritzville fine sandy loam artificially infested as previously described (17) with *F. s.* f. sp. *pisi* at 500 colony-forming units per gram (cfu/g) of soil or *P. ultimum* at 300 cfu/g plus *F. s.* f. sp. *pisi* at 500 cfu/g. Uninfested soil was included as a control. Twenty-one days after transplanting, plants were harvested and leaf area, root weight, and disease index were determined. Disease index ratings were 0–5. A value of 5 indicated a dead plant, and a disease index of 0 was assigned to plants completely devoid of root rot symptoms. There were five replications of each treatment with five plants per replication. Data were analyzed using ANOVA and treatment means separated with Duncan's multiple range test.

Fig. 1. Symptoms of hollow heart. Compare healthy cotyledon at left with C3 seed in center and C1 on right.

RESULTS

Electroconductivity and hollow heart. For all cultivars tested there was a significant linear relationship between hollow heart severity category and EC (Table 1). In nine of the 12 varieties tested, 70% or more of the variation in hollow heart severity was accounted for by the observed differences in EC (R^2 range 55–89). In general, the mean EC reading for a lot increased as the percent healthy seed decreased, but this was not consistent. The expected EC values from the regression equation for healthy seed were quite variable, ranging from 35–61 μamps; however, separation of healthy seed from those with severe hollow heart (C3) was clear,

TABLE 1. Relationship between hollow heart severity of affected pea seed and electroconductivity (EC)

Cultivars[a]	Healthy and D1 seed in seed lot (%)	Mean EC reading[b]	R^{2c}	Predicted EC value (μamps)[d] Healthy	Severe hollow heart (C3)
OSU-605	18	67 ± 2	0.88	47	84
Dual	17	67 ± 2	0.79	44	75
Canners 24	21	95 ± 2	0.79	61	108
I-686	41	76 ± 1	0.86	56	93
Preperfection	50	60 ± 2	0.70	47	86
79-123	51	66 ± 2	0.81	49	86
79-152	51	46 ± 3	0.62	37	68
Bolero	69	45 ± 1	0.70	35	69
Fr-G24	72	60 ± 2	0.89	52	84
80-1336	83	56 ± 1	0.55	46	88
Stampede	89	39 ± 1	0.67	37	68
FR-2318	75	46 ± 2	0.76	36	75

[a] Cultivars were selected on the basis of percent hollow heart as determined from a previous study (17).
[b] Mean EC readings for all seed of each cultivar plus or minus standard deviation.
[c] Coefficient of determination for regressions of hollow heart severity category 1–6 on the mean EC reading of seed in each hollow heart category.
[d] Predicted EC values based on linear regression equations for each cultivar.

with the predicted value for healthy seed averaging 36 μamps less than severely affected seed (C3) over all varieties tested.

The distribution of hollow heart severity categories for cultivar OSU-605 within individual EC categories is shown in Table 2. At each successive EC category the percent healthy and D1 seed decreased, whereas the percent of C2 and C3 seed increased. Although 68% of the seed in the 25–50 μamps EC category were either healthy or D1, only 11% of all seed tested fell into that EC category. Four percent of the seed in the 70–90 μamps EC category were healthy or D1, suggesting that factors other than hollow heart also affected EC.

Growth studies. Percent and rate of seedling emergence were greatly influenced by the EC category from which the seed were taken (Fig. 2). Seed from all categories except the 91–200 μamps achieved greater than 90% emergence; however, the rate of emergence for seed in the 70–90 μamps category was slower. Maximum emergence of seed in the 91–200 μamps category was 44%. Seed germinated but failed to emerge because of seed rot. Many of the seedlings in this category stopped growing soon after emergence.

The relationship between EC category means in μamps and growth parameter mean was very strong, as indicated by linear regression (Table 3). For every parameter measured, the difference between the first two EC categories was minimal. However, the decrease in plant growth in the 70–90 and 91–200 μamp categories was dramatic.

Hollow heart and susceptibility to root rots. Seedlings affected by hollow heart were smaller and more susceptible to root rots than healthy intact seedlings (Table 4). In every instance, seedlings with hollow heart had greater leaf area and heavier root systems than seedlings with both cotyledons removed, but less than seedlings with only one cotyledon removed. The differences were not always significant. Removal of both cotyledons was the most severe seed treatment and resulted in significantly smaller plants than whole seed controls.

In soils infested with *F. s.* f. sp. *pisi*, hollow heart seedlings had a significantly higher disease index than any of the other three seed treatments. In soils infested with both *F. s.* f. sp. *pisi* and *P. ultimum*, seedlings with hollow heart and those with both cotyledons removed had significantly higher disease indices than the whole seed control. All plants growing in soil infested with both pathogens were extremely stunted compared with seedlings in soil infested with *F. s.* f. sp. *pisi* or uninfested soil. Although seedlings with hollow heart were smaller and more susceptible to infection by *F. s.* f. sp. *pisi* as indicated by the disease index, increased disease severity was not sufficient to reduce plant growth. The differences in leaf area and root weight between hollow heart seedlings growing in soil infested with *F. s.* f. sp. *pisi* or uninfested soil were not significant. The extreme disease severity and reduction in growth of plants growing in soil with both pathogens was primarily a result of infection by *P. ultimum*. The severity of the infection was more likely caused by transplant trauma than any soil or seed treatment, since it occurred over all treatment combinations.

DISCUSSION

The general consensus from previous studies is that EC readings do not accurately reflect the potential for hollow heart in a given seed lot or variety, and that hollow heart is only marginally related to EC (3,12,18). These opinions were based on studies in which EC readings were made on bulked seed and no differentiation was made between degrees of hollow heart. When one looks at the average EC readings for the 12 varieties in this study (Table 1), similar conclusions could be made. However, when seed with hollow heart were separated into categories of increasing severity and individually analyzed, a significant correlation between EC and hollow heart was found. In addition, although there was considerable variation in the EC readings of healthy seed among the different varieties, in most cases severely cavitated seed were easily separated from healthy seed solely on EC readings. Therefore, if a given seed lot is known to have been predisposed to hollow heart then EC readings on individual seed can, with considerable accuracy, aid one in estimating the potential for severe hollow heart in that lot. The results presented here support this statement. When seed known to have been predisposed to hollow heart were separated into four EC categories, the seedlings

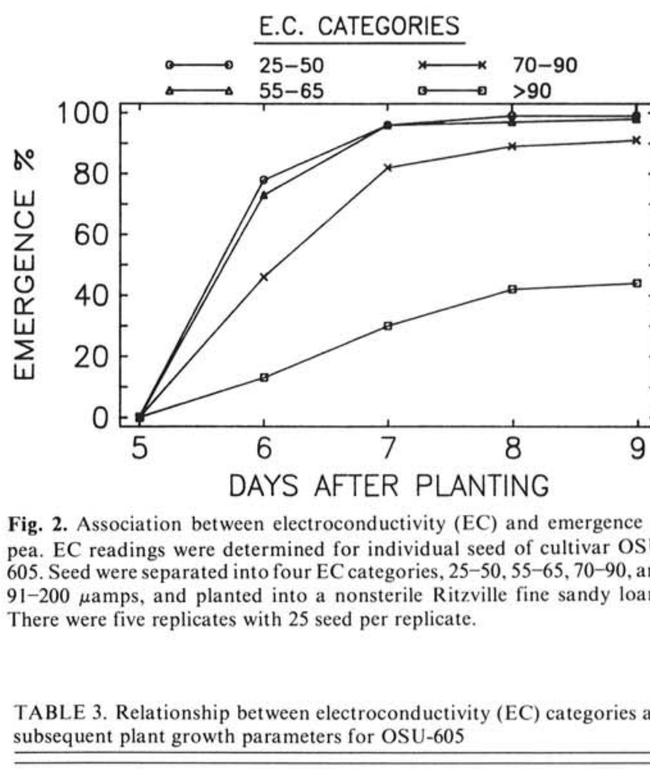

Fig. 2. Association between electroconductivity (EC) and emergence of pea. EC readings were determined for individual seed of cultivar OSU-605. Seed were separated into four EC categories, 25–50, 55–65, 70–90, and 91–200 μamps, and planted into a nonsterile Ritzville fine sandy loam. There were five replicates with 25 seed per replicate.

TABLE 2. Incidence and distribution of seed from varying hollow heart severity categories within electroconductivity (EC) categories

	Hollow heart (%)[b]			
	EC categories (μamps)[c]			
Hollow heart[a] categories	25–50	55–65	70–90	91–200
H + D1	68 ± 11	39 ± 17	4 ± 2	0
D2 + C1	27 ± 18	41 ± 17	18 ± 7	1 ± 3
C2 + C3	5 ± 11	20 ± 10	78 ± 10	99 ± 3

[a] Germination seed were separated into hollow heart categories 6 days after EC readings were taken (see text for explanation of categories).

[b] Values represent the mean percent and standard deviation of hollow heart seed present in each EC category. When hollow heart categories were combined the total percent seed from all replications in each EC category was 11, 29, 43, and 17% in the 25–50, 55–65, 70–90, and 91–200 EC categories, respectively.

[c] Electroconductivity was determined on steep water of individual seeds after a 24-hr soak in glass distilled water. There were five replications of 100 seed.

TABLE 3. Relationship between electroconductivity (EC) categories and subsequent plant growth parameters for OSU-605

EC categories[a] (μamps)	Plant ht (cm)	Dry top wt (g)	Leaf area (cm^2)	Dry root wt (g)
25–50	6.58	1.05	27.0	0.11
55–65	6.52	0.99	24.6	0.10
70–90	4.74	0.66	16.9	0.07
91–200	1.34	0.14	3.8	0.02
R^{2}[b]	0.96	0.97	0.98	0.98

[a] Electrical conductivity was determined for individual seed of variety OSU-605 after a 24-hr soak period. The seed were then separated into four EC categories. There were five replicates of 25 seed each. Seedlings were harvested 3 wk after planting in the greenhouse.

[b] Coefficient of determination for linear relationship between EC category means and growth parameter means. All regressions were significant at $P < 0.001$. The regression models for each parameter were as follows: Plant ht, $y = 9.0 - 0.052X$; dry top wt, $y = 1.43 - 0.009X$; leaf area, $y = 36.3 - 0.226X$; and ary root wt, $y = 0.14 - 0.001X$.

TABLE 4. Effects of hollow heart and cotyledon removal on disease index, leaf area, and root weight[x]

Cotyledon treatment	Disease index[y]			Leaf area (cm^2)			Dry root wt (g)		
	Uninfested	Fsp	Fsp + Pu	Uninfested	Fsp	Fsp + Pu	Uninfested	Fsp	Fsp + Pu
Severe hollow heart	0.4 aA[z]	1.6 aB	3.8 aC	102 abA	94 bcA	67 abB	0.49 bA	0.46 bA	0.27 abB
Both cotyledons removed	0.3 aA	1.0 bB	4.0 aC	86 bA	77 cA	49 bB	0.41 bA	0.41 bA	0.23 bB
One cotyledon removed	0.4 aA	1.1 bB	3.6 abC	116 aA	105 abA	78 aB	0.52 bA	0.49 bA	0.35 abB
Whole seed	0.1 aA	0.9 bB	3.2 bC	113 aA	114 aA	85 aB	0.68 aA	0.64 aA	0.38 aB

[x] Peas were grown in a nonsterile Ritzville fine sandy loam infested with *Fusarium solani* f. sp. *pisi* (Fsp), 300 cfu/g soil; *F. s.* f. sp. *pisi* and *Pythium ultimum* (Pu), 300 cfu/g soil or uninfested.

[y] Plants were assigned a disease index value between 0 (no disease symptoms) and 5 (dead plant).

[z] Small letters are for within column comparisons and large letters are for comparisons within rows. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

in the two highest categories grew very poorly (Table 3). When seed from the same lot were evaluated for hollow heart, 78% in the 70–90 μamp category and 99% in the 91–200 μamp category had severe C2 and C3 hollow heart (Table 2). These results indicate the possibility of using EC readings on predisposed seed to predict the percent severe hollow heart in a seed lot after the initial destructive sampling has been done. Using EC readings, one could, with a high degree of confidence, select seed varying in hollow heart severity. This nondestructive sampling technique could be very useful in researching hollow heart, especially in evaluating its effects on growth and yield parameters in the field.

The key to successfully using EC readings to evaluate hollow heart and effects on seedling growth is the separation of hollow heart into severity categories. If one looks at the results in Tables 2 and 3 as an example, the majority of seed with EC readings >70 were either C2 or C3. These seed produced plants that were significantly smaller and weaker than plants in the two lower EC categories where most of the seed were C1 or healthier. Likewise with emergence, seed in the 91–200 μamp EC category were 99% C2 or C3, and percent emergence was greatly reduced. Failure to differentiate between levels of hollow heart severity could explain why there has been so much disagreement in studies evaluating the effects of hollow heart on emergence and growth parameters. Without using a disease index or in some way estimating disease severity, similar problems would occur when working on root or foliar diseases.

In evaluating the effects of hollow heart on disease susceptibility we used only C2 and C3 seed to compare with healthy seed. Although direct evidence has not been obtained for the effect of hollow heart on yield of field grown peas, numerous studies (8,9) have shown that weakened seed produce seedlings that are more susceptible to soilborne pathogens and ultimately produce reduced yields. The results reported herein and others (3–5) indicate that hollow heart reduces plant growth and increases susceptibility to root rot pathogens under greenhouse conditions. Although seedlings with severe hollow heart had a significantly higher disease index than whole seed controls, the increased disease severity was not sufficient to significantly reduce plant growth. The plants were harvested at an early age, however, and previous studies (17) indicate that low inoculum densities of *F. s.* f. sp. *pisi* will not adversely affect plant growth until later in the plant's development. If the plants in this study had been harvested at bloom, differences caused by increased Fusarium root rot might have been significant. Because hollow heart is associated with increased seed electrolyte loss and reduced growth and vigor of peas, it is reasonable to expect plants from affected seed to be more susceptible to certain root rots and yield less in the field.

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